INFLUENCE OF NUTRITION IN EXPERIMENTAL INFECTION[1]

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"What is one man's meat is another man's poison" (1) is demonstrated daily in every medical clinic. Although clinical observations as well as general impressions suggest that optimum nutrition commonly aids resistance, our knowledge of nutrition is still too fragmentary to give an adequate statement of "optimum nutrition" for persons living under markedly diverse conditions "from Greenland's icy mountains to India's coral strand," and our knowledge of the factors involved in resistance seems even less complete. Even though we can suggest diets for various living conditions with a fair degree of success, the world is far from providing even an approximation of these diets to millions of the members of our family. Malthus is riding hard and fast with all four of the horsemen of the Apocalypse.

It is recognized that what appears to be abundant health under apparently admirable living and dietary conditions, does not protect against the common diseases of childhood, measles, chickenpox, or mumps, nor against many diseases of adult life such as the common cold and influenza. Ill health and poor nutrition do, however, appear to increase the incidence of such diseases as rheumatic fever and tuberculosis.

[1] This review stems from a "Round Table" held at the annual meetings of the Society of American Bacteriologists at Minneapolis, May 1948.

Cyrus P. Barnum, Jr. and David Glick, both of the Department of Physiological Chemistry of the University of Minnesota Medical School took part in the discussion but did not present papers and are not therefore involved in this review.

Paul F. Clark was the convenor of the "Round Table" and has served as editor of these papers.

What then is the evidence pertaining to the influence of nutrition on susceptibility or resistance in the more adequately controllable experimental infections? We shall interpret the term nutrition quite broadly, to include not only the utilization of commonly recognized foodstuffs, but also any chemical compounds which are known to affect cellular metabolism. Our consideration will necessarily be incomplete and devoted chiefly to the results of recent studies. With surprise at our own temerity in tackling so complex a problem, may we suggest certain "ground rules" which we would lay down for ourselves and which we should hope might be followed by all persons working in this field.

1. The fundamental importance of the genetic constitution both of the host and of the parasite have frequently been overlooked; we would stress the necessity of considering these genetic factors in most animal experimentation. We would suggest that universities combine in building up stocks of all the common experimental species of known genetic constitution. Each animal would cost more but the quality of the results would be markedly improved.
2. The choice of experimental model is always difficult and never free from objection. A disease spontaneous in the species under study is usually preferred; frequently, however, if the parasite from man can be transferred to another species with the production of a disease somewhat similar to that in man, this becomes a favored model, for example, Lansing strain poliomyelitis in mice.
3. The natural portal of entry can rarely be employed although if possible it is preferred over an artificial route. Frequently the route chosen must be highly artificial involving more or less trauma. The results from different routes of injection may be compared, but never directly.
4. In deficiency experiments, basal rations of known chemical composition should be used if possible, with varying levels of the particular component under study and with especial attention to an adequate intake of all essential factors except those deliberately restricted. With low levels of the factor, possible competition between host and parasite must be considered.
5. Not infrequently, the animals on experiment are suffering from complex deficiencies, dehydration, etc., at the time of the challenge infection. The distinction should be maintained between inanition, a quantitative depletion due to ingestion of an insufficient amount of an otherwise adequate diet, and malnutrition in which the caloric intake may be adequate but qualitatively a deficiency of one or more essential dietary components exists.
6. Age, weight, sex, segregation of litter mates, physical condition of the cages, likelihood of coprophagy, variation in intestinal flora, temperature and humidity of the laboratory should be and usually are well appreciated at the present time.
7. Consideration must be given to reports on dietary influence on protein synthesis, antibody formation, phagocytic activity, etc.
8. Manifestly, experimental groups of animals should be sufficiently large to provide statistically significant figures. A consistent tendency in several smaller series may, however, be even more convincing.

9. Uninfected animals on the deficient diet and a group injected with the uninoculated medium or normal tissue are controls too frequently omitted.
10. Since nutrition studies commonly extend over a long period, the virus or other parasites should as far as possible be frozen, or frozen and dried in quantity to serve as a basis for fresh material in the continuing experiments. Too many alterations in virulence or other characteristics may occur if serial passage virus or repeated transfer cultures are employed.

THE GENETIC BASIS OF NUTRITION EFFECTS IN NATURAL RESISTANCE TO INFECTION

Howard A. Schneider

In the understanding of how nutrition may influence an infection, we are, I believe, all agreed that nutrition is logically considered an environmental factor, and by introducing the necessity of dealing with genetic constitution, or the influence of heredity, those tired and venerable ghosts which plague every biological banquet, Heredity and Environment, immediately rise to their feet. It is our purpose, if possible, to show that these are not two antagonists, but only two aspects of a single phenomenon, the Natural World.

First, may I present evidence which leads us to begin a discussion of nutrition with facts about genetics. In the natural world infectious disease is a population problem. As our methods of study have improved we have become increasingly aware that the pathogens, like the poor, are with us always. It is also true, and a matter of direct observation, that when infectious disease makes it appearance, only certain members of the population are affected while others, in apparently the same circumstance, remain unaffected. Why is this so? The experimental epidemiological studies of Topley, Greenwood and Wilson in London and Webster in New York answer this question in part. Using herds of mice and natural diseases of mice these two groups sought by direct experiment to unravel the events in epidemic phenomena. The London School made many important contributions which it would be an impertinence to cite here in view of the detailed summary that group has published (2). Our present necessity, however, may drive us to say that, by and large, the London School concerned itself with the epidemiological consequences of the spatial and temporal arrangements of hosts and pathogens, and their studies demonstrated the important influence on epidemic disease of such factors as population densities, host immigration rates, the assembly and dispersal of herd units, etc. This spatial-temporal analysis, although it helped to formulate, and demonstrated experimentally, certain epidemiological concepts, did not, in my opinion, fully answer the fundamental question of the diversity of disease response in an exposed population. It was Webster who successfully faced the question of survivorship and made an analysis which applies here. Why, in these herds, did certain mice die and others live? Is it because of unequal dosage and hence different risks of exposure? No. Webster showed that identical doses, directly administered, produced the same diversity in disease effects (3). Is it because some mice first encountered, by chance, a "small immunizing dose" and thus were able to survive the subsequent, larger, average dose experience of the herd? No. In direct experiments with

mice and mouse typhoid Webster (4) was never able to demonstrate the reality of the "small immunizing dose" (For an opposite view see Greenwood *et al.*, 2.)

Webster turned to the hypothesis that the variation in the disease response was due to variation among the mice. This is a fundamental recognition that the description, "a herd of mice," is an inadequate one to be applied to the host in experiments in infectious diseases. But if the mice varied, what was the cause of that variation? Heredity? Environment?

Webster elected to examine the case for heredity. By inbreeding and selecting from a common stock of mice he was able to extract two strains of mice which were widely divergent in their response to infection with *Salmonella enteritidis*, one strain exhibiting almost complete survivorship, the other almost complete mortality (5). By performing this basic operation of the geneticist, inbreeding and selection, Webster was able to arrange the hereditary material and perpetuate stocks of mice which were, from generation to generation, predictably resistant or susceptible, i.e., resistance and susceptibility were *capable* of being brought under genetic control. Tested under herd conditions, by assembling herds of different percentile compositions of these two stocks, these relationships held true. Resistant stocks survived and susceptible stocks died (3).

We must bear in mind the operation, inbreeding and selection, which was performed to obtain these stocks. Those finally obtained serve to demonstrate, not that resistance and susceptibility are always or necessarily under genetic control, but rather that, if we do as the geneticist does, we may bring them under control which we label genetic.

In later work, Webster showed that resistance or susceptibility to mouse typhoid was independent of resistance or susceptibility to a second experimental infection, St. Louis encephalitis (6). This independence made possible the arranging of four different stocks of mice illustrating the four possible combinations of resistance or susceptibility to two different diseases. The status of these stocks is evident from tests performed in 1942 (7) and presented here in table 1. I believe that this demonstration decisively demolishes any notion we might have of resistance, as an attribute of "health," to all infectious diseases. In one and the same animal we may have resistance to one disease and susceptibility to a second disease.

We have emphasized that the demonstration of the genetic factor does not thereby exclude the operation, and even importance, of other factors. We must, however, grant genetics a position of primary importance and we maintain that this fact is frequently neglected in experimental studies. The long time operation of the selective effects of disease tends to drive the control of these events into the germ plasm, so to speak, and thus to escape the vicissitudes of the environment. If, by evolution, this process is complete, we recognize the *fait accompli* and do not waste our energies further. We are not, for example, considering the effect of nutrition on the occurrence of blue or brown eyes, or of blood groups. In the case of the infectious disease, the wide range of variation so often met in the disease response indicates, I believe, that the evolutionary process is still in

progress. Therefore, we need not throw up our hands and resign all to the geneticist. But neither should we ignore the fact that at least some of the evolutionary process has preceded us in time. No, if there is any hope that the slow grinding mill of evolution has not yet ground all the meal, we must recognize that as nutritionists, as environmentalists, we must deal with the situation as genetics has left it at this spot in evolutionary time. This is why genetics occupies so pivotal a position among the biological sciences.

Before we leave this brief description of the role of genetics in infectious disease, I should like to mention that whenever the influence of genetics has been analyzed in human populations with appropriate techniques, its role has been made evident. This is true for infectious disease of such diverse etiology as leprosy (8, 9), rheumatic fever (10, 11), poliomyelitis (12), and tuberculosis

TABLE 1‡

Survivorship of inbred strains of mice selected for resistance or susceptibility to infection with S. enteritidis or St. Louis encephalitis virus

DESCRIPTION OF STRAIN	STRAIN DESIGNATION	TEST PATHOGEN	NO. OF MICE TESTED (IN 1942)	SURVIVORSHIP*
				per cent
Bacteria-resistant, virus-resistant	BRVR	*S. enteritidis*	449	92
		St. Louis encephalitis	606	76
Bacteria-susceptible, virus-resistant	BSVR	*S. enteritidis*	86	2
		St. Louis encephalitis	98	88
Bacteria-resistant, virus-susceptible	BRVS	*S. enteritidis*	287	82
		St. Louis encephalitis	519	2
Bacteria-susceptible, virus-susceptible	BSVS	*S. enteritidis*	285	2
		St. Louis encephalitis	271	3

* Testing dose: *S. enteritidis*, 5,000,000 by stomach catheter; St. Louis encephalitis, 10^{-2} suspension of infected mouse brain, intranasally.

‡ Adapted from Schneider and Webster (7).

(13, 14). One of the best of these studies is that of Kallman and Reisner (14) in tuberculosis. Table 2, taken from these authors, shows in summary form the effect of blood relationship on tuberculosis morbidity as studied by the twin method. These data comprise one of the most telling arguments to bring to our full attention the genetic factor in infectious disease. For example, if one member of a twin pair over 14 years of age develops clinical tuberculosis, then the chances are 2–3 out of 10 that the other twin will develop tuberculosis if the second twin is a dizygotic co-twin, *i.e.*, non-identical. But, if the twin relationship is monozygotic, *i.e.*, identical, then the chances of the co-twin developing tuberculosis rises to 9 out of 10. These are sobering facts.

If we grant the fundamental importance of genetics, but are insistent that we ought at least try to examine the possible influence of nutrition, we can make a beginning by considering the various genetic possibilities, both for host and

TABLE 2

Tuberculosis morbidity rates in the twin index families

	GENERAL POPULATION* OVER FOURTEEN YEARS OF AGE	RELATION TO TUBERCULOUS INDEX CASES					
		Husbands and wives	Parents	Half-siblings	Full siblings	Dizygotic cotwins	Monozygotic cotwins
Number of cases							
Cases of tuberculosis.....	—	14	114	4	136	42	48
Rates of reference							
Uncorrected.....	—	226	688	42†	720†	230	78
Corrected.....	—	197.5	676	33.5	534	164	55
Morbidity rates							
Uncorrected for differences in age.....	1.08	6.2	16.6	9.5	18.9	18.3	61.5
Corrected (Weinberg method)..	1.37	7.1	16.9	11.9	25.5	25.6	87.3

* Estimated for a population in which the ratio of white to nonwhite persons approximates 5:1.

† All individuals above the age of fourteen.

Courtesy of Kallman, F. J. and Reisner, D., 1943, *American Review of Tuberculosis*, **47**, 549.

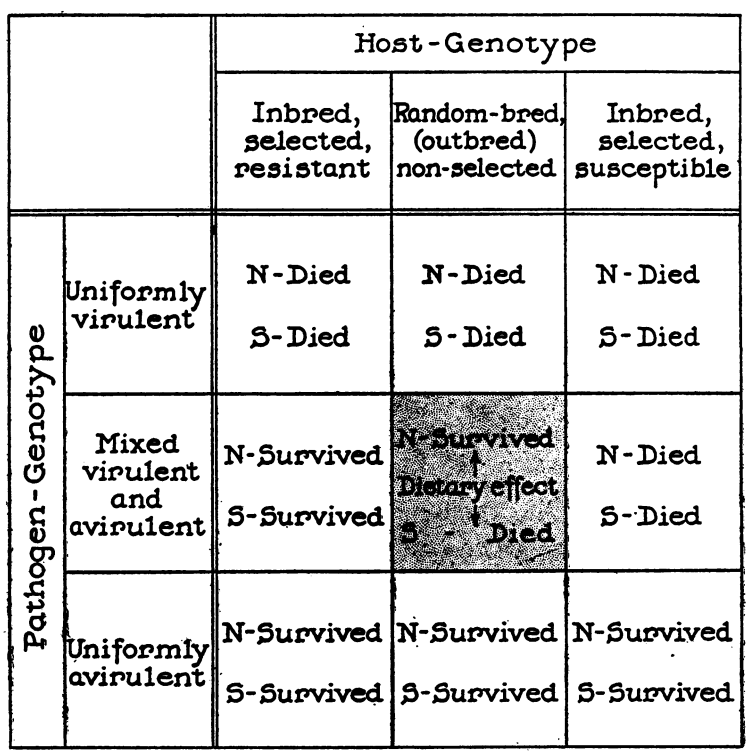

FIG. 1. The effect of a natural (N) and a synthetic (S) diet on survivorship following infection in nine different genetic circumstances.

From H. A. Schneider, Transactions of the New York Academy of Medicine, 1948 Eastern States Health Education Conference. (By permission.)

pathogen, under which nutrition might work. If we do this (7, 15, 16), we find that we can distinguish three genetically different kinds (genotypes) of hosts and three genetically different kinds (genotypes) of pathogen populations. Thus hosts are either (*a*) inbred (genetically uniform) and resistant, (*b*) inbred (genetically uniform) and susceptible, or (*c*) outbred (random-bred, genetically heterogeneous) and non-selected. Pathogen populations are also composed either of (*a*) uniformly virulent particles, (*b*) uniformly avirulent particles, or (*c*) a heterogeneous mixture of both kinds of infectious particles. In these statements we can see the attempt to encompass the facts of genetic variation. We can also see that host and pathogen can meet in 3 × 3 or nine different kinds of meetings. In which of these circumstances can we affect the mortal outcome of the infection by manipulating the nutrition of the host?

Before we attempt to answer that question, we are faced by the necessity of deciding what kind of nutritional manipulations we choose to make. The number we have to choose from is enormous, but let us follow the historical precedent. Let us inquire whether in the gap between present day knowledge of nutrition and the world of natural foodstuffs there may exist entities which our tests will reveal.

So if we compare (7, 15, 16) a diet of natural foodstuffs, whole wheat plus whole milk with a synthetic diet which contains all the desirable compounds we know about, and make this comparison in the nine genetic instances we have discussed before, we shall have at least one answer to our question. In figure 1, for mice and mouse typhoid, this answer is presented. I could go on to tell you about this survivorship principle revealed in this ninth instance, and failing to be revealed in the other eight, how it is present in the germ of wheat and go on to list its chemical properties as we have learned them thus far. For the present, however, we are content to make this point—that in the field of infectious disease, nutrition of the host operates in a genetic framework and the area of its operation is definable as the area in which genetically heterogeneous hosts meet with genetically heterogeneous pathogen populations.

THE INFLUENCE OF NUTRITION ON RESISTANCE TO EXPERIMENTAL INFECTIONS WITH HELMINTH AND WITH PROTOZOAN PARASITES

William Trager

The experiments of Ackert, McIlvaine and Crawford (17) with the intestinal nematode *Ascaridia lineata* of the chicken were among the first to indicate a relationship between a specific dietary factor in the nutrition of an animal and the degree of resistance of the animal to an infectious agent. When 7 week old chickens were placed on a diet deficient in vitamin A, they showed definite signs of deficiency 3 weeks later. If these chickens and non-deficient control birds receiving the same diet supplemented with cod-liver oil were then infected with counted numbers of eggs of *A. lineata*, the deficient chickens subsequently showed more and larger worms than the non-deficient ones. Weakened peristalsis and the larger amount of feces present in the intestines of the deficient chickens, thus perhaps providing a better food supply for the worms, were suggested as possible

explanations for the observed effects of the deficiency. Within the next few years, instances were reported in which vitamin A deficiency interfered with the development of acquired immunity in rats to the intestinal nematode *Nippostrongylus muris* (18), with the resistance of rats to both initial and secondary infection with *Trichinella spiralis* (19), with the resistance of dogs to two species of ascarids (20), and with both the natural and acquired resistance of rats to the nematode *Strongyloides ratti* (21). McCoy (19), observed that rats on a vitamin A deficient diet showed a lowered resistance to infection with *Trichinella spiralis* several weeks before the appearance of any other signs of avitaminosis. While in normal rats adult trichinae live only two weeks, in the vitamin A deficient rats they survived as long as their host—up to 57 days. Lawler (21) found that the resistance to strongyloides infection of rats on a vitamin A deficient diet was not decreased until the stores of vitamin A in the liver had been completely depleted. When this condition had been reached, resistance both to primary and to secondary infections with *S. ratti* was significantly lowered.

Deficiency in either thiamine or riboflavin lowers the resistance of rats to *Nippostrongylus muris*, another nematode parasite (22). The effect was slight in a primary infection but marked in a superinfection, suggesting that the deficiencies interfered primarily with antibody formation. Protein deficiency also interferes with the development of acquired immunity to this parasite (23).

Larsh (24) found that increased development of the cysticercoids, or larval forms, of the tapeworm hymenolepis occurred in mice which were partially fasted or were given alcohol. If the fasted or alcoholic mice were given a vitamin mixture, their resistance was as great as that of control normally fed mice, although their food intake was as low as that of fasted or alcoholic mice not given the vitamin supplement.

Much of the experimental work on nutrition and resistance to protozoan infections has been done with representatives of two genera of parasitic protozoa, the genus *Trypanosoma* which typically develops within the fluid portion of the blood of its host, and the genus *Plasmodium* which undergoes a major portion of its developmental cycle within the red blood cells of its vertebrate host. Manifestly, these two genera include some of the most important infectious agents of man and domestic animals, members of the genus *Plasmodium* being responsible for malaria, and the trypanosomes for African sleeping sickness and Chagas' disease of man and for nagana and related diseases of cattle and horses.

With the protozoa, as with other types of infectious agents, two opposite effects of nutrition on resistance are possible, and both have been observed. Let us consider first those instances in which a nutritional deficiency rather unexpectedly appears to enhance resistance to a protozoan parasite. Reiner and Paton (25) reported that rats on a diet deficient in the vitamin B complex survived infections with the blood flagellate *Trypanosoma equiperdum* slightly longer than did control animals on an adequate diet. Becker (26) observed that rats fed a heated diet and infected with the intestinal coccidian parasite *Eimeria nieschulzi* excreted in their feces fewer oöcysts than did rats which were fed the

same diet but not heated. The number of oöcysts excreted was increased, indicating a more severe infection, if the heated diet was supplemented with pantothenic acid. Similarly, if rats were fed a poor diet and were infected with eimeria, a supplement of calcium pantothenate did not improve the growth of the rats, but it did favor the growth of the parasites, as evidenced by increased oöcyst excretion (27). The addition of pyridoxine alone had a similar effect, but if thiamine was added together with the pyridoxine even fewer oöcysts were produced than in controls fed the unsupplemented diet (28). The feeding of thiamine together with pantothenate, however, had no such effect on the growth-promoting potency of pantothenate for eimeria.

Calcium pantothenate favors the survival of a bird malaria parasite (*Plasmodium lophurae*) in suspensions of duck or chicken red blood cells maintained *in vitro* (29). The results suggest that a deficiency of the host in patothenic acid may affect adversely the development within it of malarial parasites. This was found true in blood-induced infections of chickens with *Plasmodium gallinaceum* (30). In these experiments the chickens were inoculated when marked differences had appeared between the supplemented and the deficient groups. Whereas the infections in the chickens receiving pantothenate reached the usual peaks with over 50% of the erythrocytes parasitized, the infections in the chickens deficient in pantothenate showed only 3% or less of the erythrocytes infected.

Riboflavin, like pantothenic acid, appears to be even more essential to the life of certain avian malaria parasites than to the life of their host (31). When chicks fed a diet low in riboflavin were infected with *Plasmodium lophurae*, they subsequently developed infections with a peak parasite number only about one-fifth as high as that reached in control chicks on an adequate diet. Chicks which were fed the adequate diet but in restricted amount, so that they grew as poorly as the chicks on the riboflavin-deficient diet, developed even heavier infection than the fully fed controls. Although riboflavin deficiency made chicks relatively unsuitable for the growth of malaria parasites, it also rendered them more susceptible, apparently, to the effects of the infection. In spite of their lower parasitemia, more deaths occurred among the infected deficient chicks than among either the infected non-deficient ones or the uninfected deficient ones.

In monkeys, a deficiency of ascorbic acid modified and greatly prolonged the course of infection with *Plasmodium knowlesi*, a malarial parasite which normally produces in monkeys an acute rapidly fatal infection (32).

Instances in which a nutritional deficiency in the host lowers its resistance to protozoan parasites are somewhat more numerous than the instances, already cited, in which the reverse has been found true. Salazzo (33) found that while normal pigeons were completely refractory to infection by *Trypanosoma brucei*, a parasite of mammals, pigeons made deficient in vitamin B complex by feeding them a diet of autoclaved wheat or polished rice showed an appreciable susceptibility to this parasite. The trypanosomes became sufficiently numerous in the blood of some of these pigeons to be demonstrable microscopically as well as by subinoculation into mice.

The resistance of rats to the blood flagellate *Trypanosoma lewisi* is markedly

lowered by deficiency in either biotin or pantothenic acid (34, 35). Except in very young rats (36), *T. lewisi* is normally a benign parasite, rarely reaching densities greater than two or three hundred thousand per mm^3 of blood and never causing death. In rats fed a diet deficient in pantothenic acid and infected with *T. lewisi*, peak parasite densities were attained 5 to 9 times as high as those seen in litter mates receiving pantothenic acid (37). In the deficient animals the parasites persisted 4 to 5 days longer than in the controls, and produced a considerable anemia. It is interesting that closely parallel effects were observed in rats which received sodium salicylate (38), a compound which antagonizes pantothenic acid in the growth of certain bacteria (39).

If rats were made moderately to severely deficient in biotin by feeding them a diet rich in egg white and were then inoculated with *T. lewisi*, they showed much heavier and more prolonged infections than did control non-deficient rats (40). Even in slightly deficient rats, significantly higher parasite densities were attained than in the controls; this suggests a specific effect of the biotin deficiency. Rats which showed marked anemia as a result of having been fed a toxic low-protein diet containing 16% linoleic acid, were nevertheless fully as resistant to *T. lewisi* as rats on an adequate diet.

The first report of the effect of a specific nutritional factor on the course of a malarial infection was concerned with the influence of biotin on susceptibility to avian malaria (41). It was found that a parasitemia of approximately twice the intensity developed in chickens or ducks made moderately biotin-deficient as compared with control non-deficient birds. This was true for *Plasmodium lophurae* both in chickens and ducks and for *P. cathemerium* in ducks. These results were soon confirmed (42).

A deficiency in folic acid (43) or in protein (44), also decreases the resistance of chickens to *P. lophurae*. Since folic acid deficiency in chickens is accompanied by anemia as well as leucopenia, the higher relative parasite counts observed may have been partly due to the smaller number of red cells. Seeler and Ott (45) reported a biphasic effect of thiamine deficiency on the course of infection with *P. lophurae* in chickens. The highest peak of parasite numbers occurred in chicks with severe thiamine deficiency, the lowest in chicks receiving the minimum requirement, and intermediate peaks in those receiving an excess of thiamine. Roos, Hegsted and Stare (46) were, however, unable to find any effect of thiamine deficiency on *P. lophurae* either in chicks or in ducks. Similarly, they found no effect either of vitamin A or niacin deficiency on the course of the infection in ducks. But niacin deficiency had an interesting effect on infection with *P. lophurae* in chickens. Peak parasite densities were reached 3 to 4 times as high as those of the controls, but the birds became free from parasites at almost the same time as the controls. Brooke (47) has described effects of poor diet in decreasing the resistance of birds to avian malarial parasites. The nature of the specific deficiencies responsible for the effects observed was not determined.

The experimental study of nutrition in relation to resistance to infection can

be approached from different angles and with different ends in view. Such studies may conceivably give an insight into the influence of nutrition on the epidemiology of human disease. They may also provide a method of investigating some of the mechanisms of resistance to infectious agents. Where a nutritional deficiency lowers the extent of growth of the parasite, and at the same time appears to enhance resistance of the host as in chickens deficient in pantothenic acid and infected with *Plasmodium gallinaceum*, it seems reasonable to assume that the lack of the nutrient renders the host a relatively unsuitable culture medium for the parasite. If the nutritional deficiency produces a leucopenia, as in folic acid deficiency, it could well interfere with phagocytosis, and a deficiency which interferes with protein synthesis could also interfere with the formation of antibody globulin and hence with that important mechanism of resistance. In fact, most workers have attempted to interpret the deleterious effects of nutritional deficiency on resistance to infection in terms of interference with the formation of antibodies of the classical immunological type. This interpretation may well be correct in some instances. It is interesting to note, however, that pyridoxine deficiency, which markedly reduces the amount of lymphoid tissue (48) and the antibody producing power of the animal, has had no observed effect on resistance to primary infection with a protozoan parasite. Protein deficiency which, as Cannon (49) has shown, lowers the ability to produce antibodies, had no effect on the resistance of the albino rat to *Trypanosoma lewisi* (40). Extreme protein deficiency, of the type which reduces the resistance of chicks to *Plasmodium lophurae* (44), might well interfere with many mechanisms other than those concerned with the synthesis of antibody gamma globulins.

Becker and Gallagher (38) have suggested that pantothenic acid deficiency or the administration of salicylates reduces the resistance of rats to *Trypanosoma lewisi* because they prevent the utilization of pantothenic acid as a coenzyme in the formation of a specific oxidative enzyme which inhibits the reproduction of the parasite. This enzyme might then correspond to Taliaferro's (50) ablastin or reproduction-inhibiting antibody.

Caldwell and György (40) found that, if large numbers of mature trypanosomes (*T. lewisi*) were injected intravenously into rats, and if the rats were then injected with hyperimmune serum which had been heated to 56 C for one-half hour, the trypanosomes rapidly disappeared, if the rats had been fed an adequate diet, but they persisted and multiplied in biotin-deficient rats. These results may indicate some abnormality with respect to complement in biotin-deficient rats. However, the hemolytic complement titer of the serum of biotin-deficient chicks did not differ from that of normally fed controls (51).

The more obvious explanations for the effect of biotin deficiency in decreasing the resistance of chickens and ducks to malaria parasites have no supporting data. Moderate biotin deficiency in these birds does not affect erythrocyte or leucocyte formation, nor is there evidence that it interferes with the production of antibodies. The plasma of these birds (as well as that of man and other animals) has been found to contain a lipoprotein which on hydrolysis yields a fatty ma-

terial having biotin activity for bacteria, mosquito larvae and chicks (52). This lipoprotein occurred at a relatively high concentration in the plasma of ducks recovering from a malarial infection and at a low concentration in the plasma of ducks about to die from such an infection. The α- and β-globulin fractions of human plasma, which are rich in this material, inhibited the growth of *Plasmodium lophurae* in suspensions of duck erythrocytes *in vitro*, whereas the fractions poor in it did not have such an effect (51, 53). On the basis of this admittedly circumstantial evidence, one hypothesis assumes that a lipoprotein of the plasma at suitable concentrations inhibits the growth of malaria parasites and contributes to their destruction by the phagocytes. Biotin is essential for the synthesis of the lipoid portion of this lipoprotein, hence a biotin deficiency would interfere with resistance to malaria. Recently direct evidence has been obtained for the existence of a substance in the plasma of mature chickens (relatively resistant to *P. lophurae*) which when injected intravenously into baby chicks (highly susceptible to *P. lophurae*) reduces the severity of their infection with this parasite.

In all studies on resistance, it is important to keep in mind the different levels at which a host may oppose attack by a parasite. Frequently resistance depends on the ability of the host to prevent the entrance of the parasite into its body. This may be accomplished by simple mechanical means, or by a chemical mechanism such as the killing of the potential parasite by the acid secretion of the stomach. It may also be brought about by an extreme cellular susceptibility, as with some fungus and virus infections of plants, where the cells initially infected die so quickly that the parasite, which requires living cells to nourish it, also dies before it can progress to the infection of adjacent tissues (54, 55). Once a parasite has succeeded in entering a host, the further course of events will depend on the suitability of the host as a source of the nutrients required by the parasite and on the ability of the host to check the multiplication or growth of the parasite. This is the level of resistance which has been dealt with in most studies on resistance to protozoan infection. The protozoa, and also some of the helminth parasites are especially well suited for studies of this type, since, as Taliaferro (56) has emphasized, with these organisms one can readily follow not only the over-all rate of actual increase in numbers or of growth, but also the rate of reproduction. One does not need to depend exclusively on relatively gross criteria such as the sickness or death of the host animal. These latter criteria depend not only on the level of resistance determined by the ability of the host to check the multiplication or growth of the parasite, but also on a third level of resistance, namely the ability of the host to overcome the various injurious and toxic effects brought about by the parasite. In infections such as malaria in which the number of parasites can be determined, it is not uncommon to observe individuals with severe symptoms, but with nevertheless an appreciably lighter parasitemia than that exhibited by other individuals who show few or perhaps no signs of the disease.

NUTRITION AND SUSCEPTIBILITY TO EXPERIMENTAL BACTERIAL INFECTIONS

L. S. McClung

In considering the older literature, I wish to quote from an extensive paper by Lassen in 1931 (57) on the course of paratyphoid infections in avitaminotic rats, particularly those deficient in vitamin A. His summary, which is perhaps typical of the period, states: ". . . every deficiency in nutrition, whether of quantitative nature or qualitative, is accompanied by an increased susceptibility to infection and a lowered resistance." With this as a point of departure, we may examine some of the claims in recent reports to see if the same summary could be made at the present.

Higgins and Feldman (58) reported that diets significantly low in thiamine and riboflavin, inadequate to provide proper somatic growth, did not influence in any recognizable manner the resistance of the white rat to intravenous injection with virulent avian tubercle bacilli; and, an abundance of vitamin C fed to guinea pigs before and after subcutaneous inoculation of 10,000 tubercle bacilli of H37 *Rv* variant did not influence the course of the disease according to Heise and Steenken (59).

Kelly (60) reported that *Spirillum sputigenum* produced local inflammation and abscesses in guinea pigs deficient in vitamin C but was non-pathogenic for healthy animals.

Badger (61) and Badger, Masunaga and Wolfe (62) found that rats deficient in thiamine were more susceptible to rat leprosy induced by a variety of routes than were control animals on an adequate diet. Rats on the deficient diet plus supplementary purified thiamine were no more susceptible than normal rats.

Saslow, *et al.* (63a) reported that monkeys on a deficient diet developed a striking granulopenic leucopenia and showed a markedly lowered resistance to spontaneous infection with high mortality. Likewise, in contrast to controls on a normal diet, these animals showed increased susceptibility to infections with *Streptococcus hemolyticus*, Group C, and influenza virus, Type A, when these agents were administered intranasally.

Several groups have studied the problem of susceptibility or resistance to pneumococcal infection. Wooley and Sebrell (64, 65) found that Swiss mice on a diet with less than minimal growth requirements of riboflavin or thiamine are more susceptible to intranasal infection with Type I pneumococci than animals receiving suficient quantities of these vitamins for good growth. Day and McClung (66), however, found no significant change in susceptibility to intraperitoneal injection of these organisms in experimental rats showing pantothenic acid deficiency compared with the normals in the same series. With mice, similarly injected and similarly deficient, no difference in susceptibility could be observed except possibly those in acute pantothenic acid deficiency. The claim of West, *et al.* (67) that pantothenic acid deficiency greatly increased the resistance of the rat to the nasal insufflation of Type I pneumococcus must be viewed with some care since only 8 animals were used. Robinson and Siegel (68)

conducted more extensive studies, also with Type I pneumococci in rats, employing 30 to 50 viable cells in a broth-mucin suspension and the intratracheal route through an incision in the neck. They reported that rats deficient in riboflavin or pantothenic acid are as resistant to experimentally induced pneumococcal lobar pneumonia as those receiving the same basal diet with adequate quantities of these factors, but that thiamine and possibly pyridoxine deficiency appeared to lower the resistance. These authors also considered the vitamin content of the tissue of the depleted animals and found sufficient quantities of the vitamins to support the growth of the microörganism. In a later report, Hitchings and Falco (69) discovered that mice on a purified diet survived the intraperitoneal injection of 100,000 lethal doses as compared with those on a commercial laboratory diet. They were unable to ascribe the difference to any known dietary essential.

With respect to infection by the salmonella, which are natural pathogens for mice, Stryker and Janeta (70) found no noteworthy difference in intestinal permeability to *Salmonella enteritidis* or of *Clostridium botulinum* toxin in rats deficient in vitamin A and control animals. Riboflavin deficient mice are much more highly susceptible to spontaneous salmonella infections than control animals according to Kligler, *et al.* (71), and likewise mice and rats in a state of avitaminosis A are more susceptible than normals (72) but with this factor included in the diet. Paired feeding experiments suggest that starvation rather than vitamin A deficiency was the more important factor. Biotin deficient rats, which were infected in groups after 14, 21, and 28 days on the diet by feeding a low dilution of a culture of *S. typhimurium* and were killed 3 days later, showed greater percentage of positive cultures of *Salmonella* from their organs than did controls. In spontaneous salmonella epidemics in mice, employing 4 week old litter mates in the groups, the deficient animals showed a higher rate of infection (73). With respect to thiamine, Guggenheim and Buechler (74) reported that deficient mice showed a markedly increased susceptibility to oral infection with *S. typhimurium* and it was suggested that with mice the diminished resistance is exclusively attributable to the thiamine deficiency, whereas the higher susceptibility of rats is due to the inanition which accompanied avitaminosis.

This short summary of claims in recent reports seems to fail to support completely the statements of Lassen given previously. In addition, in order to keep this summary within appropriate limits, we have refrained from pointing out, for each report, factors which might cast doubt on the validity of the conclusions suggested by the authors. In many, if not all, of the reports, serious criticism could be made of one or more of the items in the experimental procedures used.

INFLUENCE OF NUTRITION IN RESISTANCE TO EXPERIMENTAL RICKETTSIAL INFECTION

Henry Pinkerton

Historically, high typhus mortality has through the centuries been associated with war and famine, and on the experimental side, certain dietary factors have

influenced markedly the response to rickettsial infection. Our experience with rickettsial infections has taught us that it may be difficult to determine whether certain compounds alter resistance by affecting the nutrition of the host or that of the invading organisms. We shall, however, consider primarily the nutrition of the host, since specific information about the nutrition of the rickettsiae is largely lacking. Our statements will be confined chiefly to typhus infection, but diseases of the spotted fever and tsutsugamushi groups appear to react in a similar manner to nutritional alterations.

Growth Requirements of the Rickettsiae. The rickettsiae are obligate intracellular parasites and have been cultivated only on this basis. Wolbach and his co-workers (81), in 1923, obtained slight multiplication of spotted fever and typhus rickettsiae in plasma tissue cultures maintained at 37.5 C. In 1931, Nigg and Landsteiner grew typhus rickettsiae in large numbers within cells in a modified Maitland culture (minced guinea pig tissue in a mixture of serum and Tyrode's solution) incubated at 37.5 C. In the same year, Pinkerton and Hass repeated the earlier attempts of Wolbach and his co-workers to grow rickettsiae within living cells in plasma tissue cultures. At 37.5 C, rickettsial multiplication was slight and transient, whereas at 32 C, massive intracellular growth of rickettsiae occured (83), and heavily infected cultures could be maintained indefinitely (84). In the Maitland type of culture, tissue cells do not multiply, but slowly die, and probably for this reason the temperature of incubation was not so important. The evidence suggested that rickettsiae did not prefer a temperature of 32 C, but grew freely in living cells maintained at that temperature because the lowered metabolic rate favored their multiplication.

This concept received more direct experimental support by the work of Zinsser and Schoenbach (85) in 1937. These authors found that in cultures of the Maitland type, incubated at 37.5 C, rickettsial growth was most active in the sixth, seventh, and eighth days of incubation, "when the tissue has ceased respiring, and is either not viable at all, or has lost much, possibly all, of its metabolic activity." This result was in striking contrast to that obtained with a typical virus (equine encephalitis) which multiplied most actively during the first two days of incubation, when tissue respiration was most active.

The oxygen tension and pH of plasma tissue cultures did not influence the growth of rickettsiae unless the alteration was sufficient to cause the death of the host cells (84). The rickettsiae of spotted fever in tissue culture, for some unknown reason, grew most freely within the nuclei of their host cells, where they formed compact clusters somewhat resembling the nuclear inclusions of viral infections (86). Typhus rickettsiae were never seen in nuclei; they were reduced in number and spherical in shape in cells which were dividing mitotically, again suggesting that an increase in cellular metabolism was unfavorable for rickettsial growth.

Zinsser and his co-workers (87) improved the Maitland medium by using Tyrode's solution with serum agar, and by spreading the minced tissue on the surface of this medium. Zia (88) grew rickettsiae on the chorioallantoic membrane of the fertile egg; and Cox (89) grew them even more abundantly in the

yolk sac. In all of these media, rickettsiae grow only within cells although, after the host cells die, many extracellular rickettsiae are found. The peculiar susceptibility of the cells lining the yolk sac of the fertile egg has not been explained. These cells are most susceptible on the fifth and sixth days of embryonic development; active multiplication takes place at temperatures between 34 and 37.5 C, and very little growth of rickettsiae occurs if the eggs are incubated at 40 C (90).

It may be assumed that rickettsiae are at least partially lacking in vital enzyme systems, and can metabolize only by diverting to their own use certain enzyme systems of the host cells. This nutritional dependence of rickettsiae on the host cells suggests that agents which modify the intracellular metabolism in the host might affect, favorably or unfavorably, the resistance of the host to infection.

Genetic Factors. Experimental typhus is normally a mild, nonfatal disease in the guinea pig and in the rat, but is fatal for certain strains of mice. The variable susceptibility of different strains of mice (91) and of fertile eggs (92) indicates again the importance of genetic differences stressed in the first section of this review.

Starvation. We have studied the effects of starvation on murine typhus infection in guinea pigs and rats (93). In male guinea pigs on a normal diet, such as oats, alfalfa, carrots, and water, the intraperitoneal injection of rickettsiae causes, after an incubation period of two to four days, marked inflammation of the scrotal sac, redness and swelling of the scrotum, and a febrile reaction lasting for several days, although the animals do not appear ill, and invariably recover. A fibrinous exudate, containing small to moderate numbers of rickettsiae is found in the scrotal sac, where the temperature is low (about 32 C), but the general peritoneal cavity remains normal. If male guinea pigs are deprived of food (but given water *ad lib.*) from the day of injection, neither scrotal reaction nor fever occur. Typhus-infected male guinea pigs which are killed after five to eight days of starvation, however, show a heavily infected gelatinous exudate in the general peritoneal cavity as well as in the scrotal sac. In starving rats injected intraperitoneally with murine typhus rickettsiae, we also found a copious exudate and a much greater accumulation of rickettsiae in the peritoneal cavity than in the control rats fed on dog chow and water.

Intoxication by benzol or by X-rays according to Zinsser and his co-workers (94) greatly reduces the resistance of rats to murine typhus rickettsiae, and causes the accumulation of rickettsiae in great numbers in the peritoneal cavity, where normally only a few rickettsiae were found. These methods, particularly the irradiation method, were used extensively to obtain rickettsiae in large numbers for carrying out immunological studies.

Vitamins. In 1931, Zinsser and his associates (95) found that a diet lacking all of the then known vitamins, carried to a point where deficiency signs developed, increased the severity of murine typhus infection in guinea pigs and rats, and caused the appearance of a pleural and peritoneal exudate with many rickettsiae. The effect which they got in guinea pigs was probably the result of ascorbic acid deficiency.

In 1939, Pinkerton and Bessey (96) reported a striking and apparently specific effect of riboflavin deficiency on experimental murine typhus infection in rats. Even in the early stages of deficiency when the rats were in relatively good condition (and had a life expectancy of three to four weeks), a complete loss of resistance was found, and the rats died in three to four days with an overwhelming infection, characterized by huge numbers of rickettsiae in all of their organs. The administration of riboflavin to such rats, even when they were at the point of death, caused complete recovery within twenty-four hours.

Vitamin A deficiency in rats, even in the late stages, did not cause appreciable loss of resistance to murine typhus (96).

Oral administration of para-aminobenzoic acid (PABA) to mice, by Snyder and his co-workers (97) and independently by Greiff and Pinkerton (92), was found strikingly to reduce the mortality from murine typhus. It is effective also against experimental spotted fever and tsutsugamushi disease. Although PABA forms part of the folic acid molecule, folic acid itself is not rickettsiostatic in the fertile egg (98).

We have injected many vitamins into typhus-infected embryonate eggs without definite effects, but it is probable that egg yolk contains adequate amounts of most vitamins. Generally speaking, vitamins are ineffective unless a deficiency exists. PABA is an exception to this statement, but it should be noted that the quantities of PABA needed to cause rickettsiostasis are enormous in comparison with the amounts required to maintain health. Recently developed knowledge of antivitamins and anti-metabolites may well prove useful in solving problems of rickettsial and viral metabolism.

Fitzpatrick (99) has found recently that rats showed an increased susceptibility to murine typhus when kept on diets deficient in the following specific ways: (*a*) low proteins; (*b*) one-tenth of the optimum supply of all vitamins of the B group; (*c*) one-twentieth of the optimum supply of pantothenic acid; (*d*) one-twentieth of the optimum supply of riboflavin; (*e*) one-twentieth of the optimum amount of thiamine. No change in susceptibility was found in rats kept on diets deficient only in containing one-fortieth of the optimum amount of pyridoxine, choline, nicotinic acid, and para-aminobenzoic acid. Susceptibility was measured by the mortality, which was usually zero in the rats on a complete diet and often 100 percent in deficient animals.

The addition of one per cent of liver powder to a complete diet did not increase resistance, but rats on a natural laboratory diet (oats, Rockland diet and fresh vegetables) were less susceptible to infection than those kept on a complete synthetic diet, even though the latter caused a greater increase in weight.

We have recently found that certain folic acid antagonists and derivatives (gamopterin, diopterin, aminopterin, and pteropterin) in maximum tolerated doses have no appreciable effect on rickettsial growth in fertile eggs (100). These compounds were injected forty-eight hours after the injection of rickettsiae.

Dyes. Methylene blue and toluidine blue, when incorporated in the diet, increase resistance and greatly lower the mortality from typhus infection in mice (101). Unfortunately, the toxicity of these drugs has made them unsuitable for use in human beings.

Other Chemotherapeutic Agents. The sulfonamides lower resistance to experimental spotted fever and typhus infection in guinea pigs (102, 103). Penicillin is rickettsiostatic against murine typhus infection in the fertile egg (104), and reduces the mortality of mice from murine typhus infection (105) from 100 percent to zero if given early, under certain experimental conditions. Parasulphonamidobenzamidine and related compounds are effective in reducing the mortality from typhus infection in mice (106). Acridine compounds are rickettsiostatic in the fertile egg (107).

Chloromycetin has been shown recently (108) to be remarkably effective against experimental infection with the rickettsiae of typhus, spotted fever, tsutsugamushi disease, and rickettsial pox. It is effective when given either orally or intraperitoneally to mice infected with tsutsugamushi disease. This compound is also effective against psittacosis. Although psittacosis is classed as a viral disease, it is biologically more closely related to the rickettsiae than to the smaller and more typical viruses.

Aureomycin (109) also is effective against spotted fever, tsutsugamushi disease, and Q-fever, both in fertile eggs and in experimental animals.

Para-aminobenzoic acid is probably ineffective against Q-fever, but streptomycin is said to be of value (110). Q-fever differs rather widely from other rickettsial infections.

Mechanisms of Action. In attempting to explain the bacteriostatic action of chemotherapeutic agents, emphasis has been placed on interference with the nutrition of the invading organisms. The intracellular location of the rickettsiae probably protects them somewhat against the direct action of most chemotherapeutic agents. The rickettsiae probably are vulnerable for a short time while they are moving from one cell to another, however, and certain rickettsiostatic agents, such as penicillin, aureomycin, and chloromycetin, may inhibit rickettsial multiplication directly in much the same way as they inhibit bacterial multiplication. We have also indications for believing that certain rickettsiostatic agents may act indirectly by altering the metabolism of the cells in which the rickettsiae multiply.

Smadel and his associates have suggested, on the basis of their observation that nucleic acid partially neutralized the action of the acridine dyes in the fertile egg, that the rickettsiostatic action of these compounds may depend on interference with an adenine-containing enzyme of the organism or host cell (107).

Lowering the temperature of infected animals is known to favor the intracellular growth of rickettsiae and the scrotal reaction, which is characteristic of many rickettsial infections in experimental animals, is undoubtedly due to the relatively low temperature of the scrotum. Typhus-infected mice, kept at a temperature of 29 to 37 C showed a survival rate of ninety percent, as compared with a survival rate of zero in parallel series of mice kept at 18 to 22 C (111).

This temperature effect makes it difficult to interpret the results of many of the experiments cited. The rapid multiplication of rickettsiae in the peritoneal

cavities of animals made ill by starvation, benzol or X-ray intoxication, and certain vitamin deficiencies may depend, to some extent at least, on a non-specific lowering of the body temperature. Zinsser and his co-workers were inclined to attribute the effect of benzol and irradiation to the destructive action of these agents on hematopoietic tissue, but this point was not proved.

The mechanism of action of temperature changes in modifying rickettsial multiplication is not clear. Phagocytosis of staphylococci by guinea pig leucocytes (112) increases with rising temperature to a maximum at 40 C, and this phenomenon may be of some importance in rickettsial infection. The importance of a low temperature (32 C) in obtaining vigorous intracellular multiplication of rickettsiae in plasma tissue cultures (83), where leucocytes are not present, suggests that temperature changes may affect rickettsial growth principally by altering intracellular metabolism.

Pinkerton and Bessey (96) believed that the loss of resistance to murine typhus resulting from riboflavin deficiency might be a direct result of lowered intracellular respiration, since riboflavin forms an essential component of an important respiratory enzyme (113).

One might be tempted to explain the rickettsiostatic action of such dyes as methylene blue, toluidine blue, and nitroacridine on the basis of their known ability to increase oxygen consumption *in vitro* (114), although it has not been shown that they have a similar effect *in vivo*.

Greiff and Pinkerton (98) have attempted to determine to what extent the rate of cellular respiration (as measured by oxygen uptake) could be correlated with rickettsial multiplication in the yolk sac of the fertile egg. An increase in the environmental temperature from 37.5 C to 40 C, which is accompanied by an increased oxygen consumption, almost completely inhibited rickettsial growth. When KCN was injected into the yolk sac, in amounts insufficient to kill the embryos, rickettsiae multiplied freely at 40 C. KCN lowered the oxygen consumption of the eggs and under certain conditions enhanced greatly the multiplication of rickettsiae. PABA, which is markedly rickettsiostatic, increased the oxygen consumption in eggs about 50%. Ortho-aminobenzoic acid and meta-aminobenzoic acid, which were not rickettsiostatic, did not increase the oxygen consumption in eggs. Under certain experimental conditions, therefore, rickettsial growth appears to be inversely proportional to cellular respiration.

The relation of metabolic processes, other than respiration, to rickettsial multiplication has not been studied. I know of no instance in which dietary deficiency has caused increased resistance to rickettsial infection, and in this respect the rickettsiae appear to differ from certain bacteria, protozoa, and viruses.

In conclusion, although only a few preliminary steps have been taken, the study of the influence of nutrition on resistance to rickettsial infection has already led to results of great interest and importance. At the present time wide gaps exist in the information which biochemists can give us regarding intracellular metabolism, and even wider gaps between the detailed knowledge of certain enzyme systems and the application of such knowledge to problems of intracellular parasitism.

THE EFFECT OF NUTRITIONAL FACTORS ON THE RELATIONSHIP BETWEEN THE BACTERIAL VIRUS AND ITS HOST

Winston H. Price

This section of the review is concerned with work done between 1943 and 1948 on the effect of various nutritional factors on the formation of bacterial viruses. It will become apparent that the relationship between a bacterial virus and its host is not a static one, but, on the contrary, is a dynamic system in which various nutrients determine the interaction between the bacterium and its virus.

Before examining the effect of various nutritional factors on bacterial virus systems, it will be profitable to state briefly the cycle of growth of the virus and the characteristics of a desirable system for studying nutritional effects. The growth cycle of the virus occurs in three steps (2): (*a*) the adsorption of the virus on the host cell, (*b*) the multiplication of the virus and (*c*) the release of the virus from the cell. During step *b*, which is called the minimum latent period, no increase in virus titer as measured by plaque counts has been demonstrated, since this method determines only extracellular virus. The virus particles are released from the cell at a time characteristic for a given virus; this produces a definite increase in virus titer. By using the one-step growth technique of Delbrück and Luria (115), one may determine whether the nutrients are involved in the adsorption of the virus to the host, or in the actual multiplication of virus, or whether the metabolite is concerned primarily with the lytic process.

Experimental evidence indicates that bacteriophage multiplication requires certain cellular reactions, but not necessarily all that are essential for bacterial reproduction (116). An appropriate system to study the effect of nutrients on virus growth would be one in which cellular multiplication did not contribute substrates for virus formation; therefore, a non-infected cell incapable of growth in the culture medium used would be an ideal host for such an investigation. Five such systems have been reported. Spizizen (116) found that cells of *Escherichia coli* suspended in a solution of 17.4×10^{-4} M glycine anhydride would support the growth of phage, although this medium would not support bacterial multiplication. Experiments by Price (117) and Krueger and co-workers (118) have indicated that staphylococcus cells would form phage under conditions where penicillin prevented any demonstrable multiplication of the cells. Herriott and Price (119) have shown that cells of *E. coli* and staphylococcus rendered nonviable by mustard gas would form phage. Finally, Anderson (120) has reported that cells of *E. coli* supported phage formation after ultraviolet irradiation although the cells were no longer capable of multiplication.

It is desirable to study those nutrients which affect virus reproduction but not the multiplication of normal cells, for if the latter is affected, it is more difficult to determine the mode of action of the nutrient. Finally, it is easier to interpret nutrient requirements and relationships in a cell which does not have too varied synthetic capacities. It should be pointed out that although an added nutrient may have no observable effect on virus formation, this nutrient substance may

be concerned in virus reproduction. The lack of effect of the nutrient may be due to the fact that the substance is synthesized by the host at a rate fast enough so as not to be a limiting substrate in virus synthesis.

The Effect of Amino Acids. Amino acids influence the formation of bacterial viruses in several interesting ways. Anderson (120) has carried out an extensive study on the effect of various amino acids on the adsorption of the virus to the host cell. He has shown that viruses T4 and T6 of *E. coli* require tryptophan for this process, that the concentration of tryptophan, temperature, pH, and length of exposure are factors in the adsorption and that the process is reversible by dilution in a synthetic medium. The degree of cofactor requirement appears to be inherited in clones of T4. Anderson has also presented evidence that coli virus T1 needs isoleucine, norleucine or methionine for adsorption, and coli virus T7, leucine, isoleucine or methionine. Although the mechanism whereby the virus becomes attached to the host cell is unknown, the finding of Anderson that certain amino acids are necessary to activate the virus before adsorption takes place may indicate that an enzyme reaction is involved in this process (120).

Cohen and Fowler (121) have found that amino acids may play a role in the formation of bacterial viruses other than a requirement for the adsorption process. Studying the formation of coli virus $T2_{r+}$ in a synthetic medium consisting of salts, ammonium lactate and ammonium chloride, they observed that the yield of virus per cell was much lower and the minimum latent period longer than on cells grown in nutrient broth. The combination of valine, isoleucine, leucine, phenylalanine, histidine, arginine, lysine, aspartic acid, glutamic acid, methionine, tryptophan, tyrosine and the purine, adenine, when added to the synthetic medium gave a burst size 90% of that found in broth and a latent period lasting 1 to 2 minutes longer. They also observed that certain amino acids decreased the minimum latent period with little effect on the yield of virus (122). For example, proline shortened the minimum latent period to a greater extent than aspartic acid, although the phage yield per cell was higher with aspartic acid. These experiments indicate that the time of liberation of the virus seems to be independent of the amount of virus synthesized.

Leucine inhibits virus reproduction, and this inhibition was overcome by the addition of valine, isoleucine or norleucine; in fact, the virus increase was greater when this inhibition was overcome than with the latter three amino acids alone. Alanine, threonine, methionine, and tryptophan had no observable effect on virus proliferation.

Spizizen (116) has reported interesting observations on coli viruses grown in glycine medium. The interpretation of these experiments is complicated by the fact that the virus-infected cells were incubated seven minutes in broth before being added to the glycine solution. Using this technique, however, he found that cells suspended in a solution of 19.5×10^{-4} M glycine or 17.4×10^{-4} M glycine anhydride were not capable of multiplication although they supported virus reproduction. Moreover, 36×10^{-4} M aminomethane sulfonic acid, the sulfonic acid analogue of glycine, inhibited virus formation in a 13×10^{-4} M glycine solution but not in a 6.5×10^{-4} M xanthine solution or in nutrient broth. Spizizen also found

that when cells of *E. coli* were kept in a solution of 13×10^{-4} M glycine for 16 days at 37 C with a trace of nutrient broth, and then transferred to a fresh 13×10^{-4} M glycine solution, they gave fairly high increases in virus. If these cells were transferred to broth, however, there was very little virus reproduction. On the other hand, cells kept first in broth for 16 days at 37 C and then transferred to a 13×10^{-4} M glycine solution formed little virus, although when these cells were added to nutrient broth a fairly large increase in virus was observed. While these latter results are difficult to interpret, they indicate how the nutritional state of the host *previous* to the infection may determine the effect of a nutrient on subsequent bacteriophage formation.

The Effect of Other Nutrients. Spizizen (123) has reported the effect of various nutrients on the formation of coli virus. The addition of yeast nucleic acid, adenosine triphosphate, glycerophosphate, diphosphopyridine, glucose-6-phosphate, and adenylic acid to a solution containing 8.7×10^{-4} M glycine stimulated bacteriophage multiplication. However, not all phosphorylated compounds were stimulatory, since fructose-6-diphosphate, glucose-1-phosphate, fructose-6-phosphate and guanylic acid were inactive. Calcium pantothentate, thiamine, nicotinic acid and pyridoxine had no phage stimulating action in the glycine medium.

Fowler and Cohen (122) have shown that an external source of carbon, nitrogen and phosphorus is essential for the reproduction of $T2_r +$ virus of *E. coli*. Cohen has interpreted his experiments to mean that in a virus-infected cell only virus material is synthesized (124). While this interpretation may be correct, it is not yet definitely established.

Experiments from this laboratory indicate that certain nondialyzable fractions from *Staphylococcus muscae* and yeast will increase the phage yield per cell of *S. muscae* in Fildes' synthetic medium containing hydrolyzed casein (125). If the cells were grown in the presence of the fraction and then centrifuged out, washed, and suspended in fresh Fildes' synthetic medium plus hydrolyzed casein, such bacteria showed an increased phage yield, if virus was added immediately (126). The active substance therefore seems to be removed from the medium by the cells. If cells previously treated with the yeast fraction were suspended in fresh synthetic medium free of this fraction and allowed first to incubate one hour, and virus then added, there was no increased phage yield. This experiment indicates that in the absence of virus, the cells convert the substance to a form not utilizable in virus synthesis. These fractions have a high concentration of ribonucleoprotein which increases as the fractions become purer. The yeast fraction isolated by Reiner and Spiegelman (127) which stimulates adaptive enzyme formation in yeast can also stimulate virus formation in the *S. muscae* system. The method of isolation and the properties of the substance isolated in both laboratories are very similar. Whether the active substance is a ribonucleoprotein and whether the two substances are the same must await the purification of each compound.

Cellular Metabolic Patterns Determining Host Resistance. In recent years, three

virus systems have been reported in which the host metabolic pattern has made them resistant to virus action.

E. H. Anderson (126) has reported that a single spontaneous mutation in *E. coli* resulted in a mutant resistant to the virus. This mutant differed from the wild type in requiring tryptophan to grow on synthetic medium consisting of inorganic salts, dextrose and ammonium chloride, and also in growing much better when a source of amino nitrogen such as asparagine was added to the medium. The mutant was resistant to T1 but not to T5. Mutants resistant to T1 and T5 and capable of growth with inorganic nitrogen and glucose were also uncovered. Another resistant mutant was isolated which required for growth both tryptophan and proline. It is not yet established whether these specific metabolic changes are directly correlated with virus resistance.

Studying various strains of virus T4 of *E. coli*, Delbrück has found that one strain required tryptophan and another strain tryptophan and calcium for reproduction. The multiplication of these virus strains is inhibited by small amounts of indole. Since the host forms indole from tryptophan, virus reproduction stops when tryptophan is added to the medium (128).

Experiments from this laboratory indicate a competition between the virus and host for nutrients. Using a staphylococcus phage, we showed that a substance present in certain acid hydrolyzed proteins was needed for virus reproduction. This factor is essential for the multiplication of the virus but not for the host, although the host may remove it from the medium. It is not needed for the adsorption of the virus to the cell. Using a high initial concentration of bacteria, a low virus concentration and a small amount of the factor in Fildes' synthetic medium, the virus will multiply for a time and then stop, although the cells, since they are not all infected, keep on multiplying. This cessation of virus reproduction results from the removal of the factor from the medium by the bacteria. Addition of more factor will result in the resumption of virus reproduction (129). Preliminary evidence of a similar competitive effect has also been observed with nicotinic acid and the nucleoprotein fraction, mentioned previously, for this staphylococcus system. Such competitive systems may play some part in the detrimental effect of the virus on the cell. If the virus, by some inhibitory mechanism, prevents the utilization of essential nutrients by the cell and utilizes these nutrients for its own synthesis, the cell will eventually die. As was pointed out earlier, Cohen (124) has also presented evidence for this view.

Influence of Nutrients on the Response of the Host to Infection. Two systems have been investigated in which nutrients have modified the normal "pathological response" of the host cells to the bacterial virus. Wahl (130) found that virus of *E. coli* needed calcium to reproduce in a synthetic medium containing ammonium sulfate, potassium chloride, magnesium sulfate, inorganic phosphate and 1 μg of thiamine per ml. Under such conditions, the host cells did not lyse. The addition of 1 μg more of thiamine per ml to the medium caused cellular lysis although the virus titer remained the same. Experiments from this laboratory have shown that with *S. muscae*, phage release is correlated with cellular lysis in nutrient broth, although in Fildes' synthetic medium the virus is released without

observable lysis (131). This latter observation has been confirmed not only by turbidity measurements but also by microscopic study. The addition of a small amount of nutrient broth to the synthetic medium resulted in cellular lysis together with the release of the virus. Cellular lysis eventually takes place in Fildes' synthetic medium, but after the virus is released from the cell. For example, in veal infusion medium, cells of *S. muscae* begin to lyse 30 to 45 minutes after infection and virus begins to be released into the medium. In Fildes' synthetic medium the release of virus from the infected cells begins after 50 to 60 minutes and the maximum phage titer is reached at about 85 minutes; the cells begin to lyse in from 90 to 110 minutes.

A further analysis of the release of virus from cells of *S. muscae* grown in Fildes' synthetic medium has recently been carried out (132). The addition of a nondialyzable fraction from yeast, resulted in a correlation between cell lysis and virus release, although the minimum latent period was the same as in the system in which the virus was released without observable lysis; that is, about 40 to 50 minutes. It should be pointed out that in all these experiments the multiplicity of infection was about 1.3. If the cells were infected with more virus particles to give a multiplicity of infection of about 6, then the cells began to lyse 40 to 50 minutes after infection and virus release was correlated with cellular lysis. In all three instances the phage yield per cell was the same. This experiment shows quite clearly how the lysis of the cells may be modified by environmental conditions and indicates that cellular lysis is an accessory phenomenon associated with bacteriophage formation.

Rather similar effects of nutritional factors have been reported with animal viruses. Thus, the Wisconsin group (133) showed that in mice with a thiamine deficiency, western equine encephalomyelitis virus multiplied and killed the host, although the animals did not show the characteristic signs of this infection. This result is quite similar to the result obtained in synthetic medium with the *S. muscae* virus system where the virus killed the bacteria and multiplied, but in which the response of the host was different from that observed in a rich veal infusion medium. Toomey, *et al.* (132) also found that mice on a thiamine deficient diet injected with the Lansing virus, died without showing paralysis. The result of these experiments may indicate that a rather specific nutritional environment is necessary for the so-called "normal" response of a host to a virus infection.

THE INFLUENCE OF NUTRITION ON EXPERIMENTAL VIRUS INFECTION

Paul F. Clark

In the field of experimental virus infection, Peyton Rous (134) made one of the earliest observations on the effect of nutrition on the transmissible chicken sarcoma. He reported that intercurrent illness of the host checked the development of the tumor and that young healthy well nourished fowl proved more susceptible than the thin and the ill.

Olitsky (135) noticed a similar response in guinea pigs inoculated with the virus of foot-and-mouth disease. Frequently the animals suffering from malnutrition or intercurrent infection showed a delayed appearance of the primary and secondary vesicles. No protective antibodies were demonstrable in the blood serum of these animals so an explanation should be sought along other lines.

Rivers (136) stated that it was a common observation in his laboratory that unhealthy or malnourished rabbits showed less reaction to vaccine virus and exhibited a lower titer to the active agent than did healthy animals.

In a series of important papers, Sabin (138) and Olitsky (139) have stressed the role of constitutional barriers which develop normally as animals pass from infancy to maturity. They have studied these changes with several viruses especially those of vesicular stomatitis and equine encephalomyelitis in mice. Several peripheral routes of infection such as nose, peritoneal cavity, muscle, or skin are readily effective in young mice and completely or largely ineffective in adult animals, depending on their age and the choice of routes. During maturation, barriers, quite unrelated to humoral immunity, develop which block the routes of dissemination possible in young animals. They have localized a number of these barriers with reasonable accuracy.

Sabin (140) has followed this line of query critically by modifying the maternal diets during the lactation period and also by weaning mice prematurely and raising these animals on selected diets. Either relative inanition or the absence from the diet of certain specific substances, such as thiamine and riboflavin during the period of rapid growth, will prevent or retard the appearance of some of the natural barriers to involvement of the nervous system. Further study of these "barriers", which quite likely may be chemical in nature, by the use of the recently developed histochemical methods might lead to further unravelling of the enigma. One is reminded of the wide variety of interference phenomena especially those induced by the vitamin analogues (Woolley, 141).

Other attempts to study host-cell virus relationships by several methods including alteration of the diet, may be seen in the experiments of Sprunt (142). After finding that under usual experimental conditions the volume of vaccinia virus injected intradermally is more important than the number of virus particles in determining infection, he carried out three types of experiments: (*a*) varying the amount of inoculum, (*b*) localizing the virus by injecting estrogenic hormones, and (*c*) increasing the spread of a constant quantity of virus by injecting the testicular spreading factor. These experiments indicated that when the virus was well localized and fewer host cells involved, the lesions were fewer.

In further studies, group *a* received no food but had free access to water, group *b* received neither food nor water, and group *c* received no food but had access to water and received in addition intraperitoneal injections of 50 ml physiologic saline solution twice daily. These regimes were maintained for ten days before virus inoculation and two days thereafter. The animals were then fed amounts sufficient to maintain weight, and group *b* was given free access to water. Plasma proteins showed no change throughout the experiments.

The animals were sacrificed seven days after vaccination and the following observations made:

1. Starvation but with access to water (group *a*) causes fewer or smaller vaccinia lesions than in controls.
2. Starvation plus dehydration (group *b*) causes even more marked decrease in vaccine lesions.
3. Starvation with increased interstitial fluid (group *c*) increases the number and size of lesions.

Sprunt suggests that the greater inhibition in group *b* may be due to the restriction of the spread of virus particles by dehydration so that fewer host cells are exposed while increased interstitial fluid as in group *c* has the opposite effect. It would seem more likely that some of the violent changes that occur during alterations in water balance are responsible for changes in cell permeability and therefore cell susceptibility.

Olitsky and Schlesinger (139) also stressed the factor of edema when they observed that hypertonic salt solution injected into the base of the tail of albino mice 3½ to 24 hours before the cutaneous injection of herpes virus, increased infection both on the basis of success with one hundred fold greater dilution of the virus and also shortening of the incubation period by one-third. Mixtures of hypertonic solution plus virus, injection of the hypertonic solution after the virus, and application of virus after the edema had subsided showed no enhancing effect. They suggested that the factors in edema which dilate the lymph vessels, render their walls more permeable, and increase the rate of flow, may bring the virus into contact with a greater number of nerve fibers in the corium and therefore aid infection.

Because of Heine's original observation, supported by several observers since his time, that poliomyelitis seems to attack the better nourished children with relatively higher incidence than the scrawny and ill fed, several experimental studies seeking knowledge in this field have been undertaken with the further hope of an increase in general information about host-cell virus relationships.

Experiments with Central Nervous System Viruses Transmissible to Mice. Foster (143) and her colleagues at Pennsylvania have, in a series of well-controlled experiments with Lansing strain poliomyelitis virus injected intracerebrally in mice, shown that a thiamine deficient diet prolongs the incubation, reduces the incidence of paralysis, and also the mortality rate, as compared with control groups on an unrestricted complete diet. Querying further the role of reduced food intake and inanition incident to thiamine deficiency, they report that the incidence both of paralysis and of death was less in the vitamin deficient group than in a paired group on a simple restricted diet with adequate thiamine. Both caloric and total restrictions were included in their experiments. They conclude that "apparently the effect of vitamin B_1 deficiency on the action of the virus is not due solely to anorexia."

A University of Wisconsin group of investigators made similar observations with essentially the same conclusions (Rasmussen *et al.*, 144). They have made several basic studies of the nutrition of the rhesus monkey, and have used a

wider variety of viruses of the central nervous system, not only Lansing but Theiler's GDVII, TO, and FA, western equine, all in mice, the virus of avian encephalomyelitis in chickens, as well as several strains of human poliomyelitis virus in monkeys (Clark *et al.*, 151), all these with a variety of diets.

In their thiamine deficiency experiments, they noted that after the usual period of observation (28 to 35 days with the polio and Theiler's viruses) when essentially all of the optimum fed controls had become paralyzed and had died, if the survivors on the thiamine deficient diet were given liberal thiamine, a number of these survivors (4 of 14 in a typical Lansing series) came down with typical paralysis. Apparently the virus had not been destroyed, but some interference had occurred so that the typical course of the disease was altered.

Also with another virus, western equine encephalitis, most mice with a severe thiamine deficiency (17 to 19 days on the deficient diet before inoculation) failed to show the characteristic signs of infection. Such mice showed only weakness progressing to atonia, tremors, and marked ataxia; the time of death was delayed somewhat but was earlier than in uninoculated deficient groups (147). Titration of brain-cord suspensions from these inoculated deficient animals, without any typical signs, showed that in this instance also the virus was not destroyed, but had multiplied apparently as well in these animals as in those that had shown typical encephalitis while fed on optimum diets. Characteristic brain lesions were also present both in the atypical deficient animals and in those with typical encephalitis fed a complete ration *ad libitum*. These histologic studies were not, however, carried out with detail adequate for a more definitive statement.

A few only of the other B group of vitamins have caused less marked alterations in the course of the disease. Pantothenic acid deficiency is especially interesting in that it gave lowered incidence of typical paralysis in the mice challenged with Theiler's GDVII, but no difference in similarly deficient and control groups when Lansing strain was employed. This suggests a difference in the requirements of the two viruses. The results with riboflavin deficiency were just the reverse, with no difference in the Theiler's FA and GDVII groups and a very slight statistical difference with Lansing virus. Through the use of a complete synthetic diet and carefully calculated salt mixtures, Lichstein (145) and his associates studied the effect of single mineral deficiencies on the susceptibility of Swiss mice to Theiler's GDVII virus. No demonstrable effect on the course of the disease was obtained by varying the level of calcium, magnesium or chlorine in the diet; a sodium deficiency, however, resulted in a small decrease in the number of paralyses; a progressively decreasing incidence of paralysis was observed as the amount of potassium or phosphorus was decreased from optimum to an essentially completely deficient level.

It should be noted, however, that although the incidence of paralysis varied from 14 to 37% in the K deficient groups as compared with 78 to 95% in K optimum groups, deaths without paralysis invariably brought the total fatalities to 100% in inoculated mice at a time when very few uninoculated deficient mice had died. This effect should probably, therefore, be designated as an alteration in the clinical picture rather than a definite increase in resistance.

Digging further into the proteins, we have employed acid-hydrolyzed casein,

with the essential amino acids in purified form, deleting one after another of the amino acids from the otherwise complete diet. The tryptophan deficient diet has given the most marked results (Kearney *et al.*, 148) with a deficiency in valine or isoleucine providing similar though less marked alterations in the clinical picture. The mice fed an optimum diet minus tryptophan showed an accelerated death rate in those inoculated with Theiler's GDVII as compared with the control deficients and a marked lack of the characteristic signs of the disease, commonly almost no cases of paralysis, as contrasted with close to 100% in groups on optimum diets. Yet in these tryptophan deficient mice, showing no signs of paralysis, in fact some of them with no signs of virus infection, the virus had multiplied abundantly as determined by subsequent brain-cord titration. The proliferation of the virus seems to have been equal to that in the optimum fed typically paralyzed animals, although more complete titrations of separate portions of brain and cord would be needed to support this statement adequately.

Many of these experiments show clearly and with reiterated emphasis that the typical clinical picture of several virus diseases can be markedly altered by a variety of dietary deficiencies. This alteration extends even to the elimination of paralysis in a high percentage of the cases but, in these altered or resistant mice, the virus continues to multiply apparently as abundantly as in animals fed a complete diet *ad libitum*. Attempts to determine with greater accuracy the nature of this interference should be pressed.

The Pennsylvania group (143), using Lansing virus, have shown that mice on a low protein diet (5% casein) exhibited a slight delay in onset of symptoms and those on low (0.4%) tryptophan exhibited a still longer incubation period, but eventually became paralyzed. The differences in their results as compared with ours may well be due to the use of a different virus and to the presence of an amount of tryptophan in the diet sufficient to provide a basis for the development of the typical disease. We have found that a diet containing as little as .05% tryptophan will not prevent paralysis in mice infected with Theiler's TO virus.

Other vitamin studies of significance include an observation of Pinkerton and Swank (146). Five per cent of 400 pigeons on a thiamine deficient diet for 5 to 12 days developed a disease similar to if not identical with psittacosis. Since the birds had seemed entirely well during a preliminary period of one to two weeks and had not been in contact with psittacosis, the authors suggested that the thiamine deficiency may have changed a latent infection with psittacosis virus into an active one.

Pinkerton and Moragues (91) have also reported a higher mortality rate and somewhat earlier deaths due to Lansing polio virus in normal mice compared with riboflavin deficient animals.

Saslaw (63) and associates observed that the absence of a nutritional factor, apparently folic acid, produced a marked leucopenia in *Macaca mulatta* rendering them susceptible to intranasal instillation of influenza virus. In normal monkeys, the virus produced only a transient leucopenia while in the nutritionally deficient monkeys with preinfection leucopenia, the virus produced marked lung involvement and death in 5 of 7 animals inoculated.

The evidence reviewed, coming as it does from many laboratories, is sufficient to make clear that nutritional deficiencies may definitely modify the usual course of several experimental virus infections. No over-all generalization can be made but the recent work brings to mind the earlier experiments of Zinsser indicating that unlike rickettsia and some other pathogens, viruses appear to thrive best under conditions of active tissue metabolism.

Manifestly, the conditions of many of the experiments are highly artificial so that one should guard against any suggestion that the facts obtained have any immediate application to the spontaneous disease involved. In most instances, however, the investigators have been studying diseases with severe infection and high mortality rates so that the challenge has been severe and less likely to detect minor differences in host-virus balance.

The interfering agents are so varied, some exceedingly simple, potassium and phosphorus, and others more complex, vitamins, vitamin analogues and metabolites in differing phases of nutrition, and some of the results so striking with almost 100% differences between controls and experimental animals, that one must urge more and continued study along many of the suggestive leads.

SUMMARY

A summary of reviews is inherently difficult. In this case, the great diversity of the experiments and the not infrequent inadequacy of controls, present such confusing and at times conflicting results that any attempt to generalize would seem premature. Mention should be made of two other recent reviews of this subject, those by Aycock and Lutman (149) and by Schneider (150). Further efforts to unravel the snarled skein should continue.

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